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Simulation of Dual Carbon–Bromine Stable Isotope Fractionation during 1,2-Dibromoethane Degradation

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Abstract

We performed a model-based investigation to simultaneously predict the evolution of concentration, as well as stable carbon and bromine isotope fractionation during 1,2-dibromoethane (EDB, ethylene dibromide) transformation in a closed system. The modelling approach considers bond-cleavage mechanisms during different reactions and allows evaluating dual carbon-bromine isotopic signals for chemical and biotic reactions, including aerobic and anaerobic biological transformation, dibromoelimination by Zn(0) and alkaline hydrolysis. The proposed model allowed us to accurately simulate the evolution of concentrations and isotope data observed in a previous laboratory study and to successfully identify different reaction pathways. Furthermore, we illustrated the model capabilities in degradation scenarios involving complex reaction systems. Specifically, we examined (i) the case of sequential multistep transformation of EDB and the isotopic evolution of the parent compound, the intermediate and the reaction product, and (ii) the case of parallel competing abiotic pathways of EDB transformation in alkaline solution.

Keywords: degradation; organic contaminants; isotope modelling; compound-specific isotope analysis; stable carbon and bromine isotopes

1. Introduction

In the last few decades, 1,2-dibromoethane (EDB, ethylene dibromide) has been frequently detected in drinking water and natural aquatic systems, due to its extensive application as an agricultural fumigant as well as a lead scavenger in gasoline [1,2]. EDB is a widespread pollutant and several studies have investigated its degradation under different environmental conditions [3–5]. However, the environmental fate of EDB is difficult to understand and to quantitatively assess since this chemical can undergo different transformation processes and its concentration distribution in aquatic systems also depends on physical processes such as mass-transfer, dilution and sorption [6–8]. Therefore, the application of compound specific isotope analysis (CSIA) is beneficial to investigate the environmental fate of EDB. CSIA techniques have been developed and applied to a wide variety of organic pollutants [9–12], for which the determination of the change of stable isotope signals could be used to identify and quantify specific transformation processes. Carbon is the most common element for CSIA applications in contaminant hydrology; however, recent developments on analytical techniques for chlorine and bromine CSIA allowed increasing applications of dual-element isotope analysis for organohalides [13–18]. Thus, different reaction pathways of halogenated organic pollutants could be characterized and understood using dual-element CSIA [19–23]. In a very recent laboratory study, Kuntze et al. [24] applied dual carbon-bromine CSIA to investigate different reaction mechanisms during EDB degradation.

In this work we propose an isotope modelling approach for dual carbon-bromine isotope fractionation based on the reaction mechanisms and the experimental data from the study of Kuntze et al. [24]. Isotope models are valuable tools to provide quantitative interpretation of isotopic data obtained during different transformation processes as well as in complex environmental systems where both physical and transformation processes influence the observed isotopic signals [25–27]. So far, isotope models have been developed and applied for multi-element isotopic prediction of

various organic contaminants, including chlorinated hydrocarbons [28–31], BTEX compounds [32,33] and organic pesticides [34,35]. However, such a modelling framework is still lacking for brominated organic compounds. This modelling case study illustrates an integrated carbon-bromine isotope modelling approach to simultaneously predict the evolution of concentration, as well as carbon and bromine isotopic signals. Our work focuses on chemical and biotic transformations of EDB with the specific goals to: (i) describe a mechanism-based integrated modelling approach to simulate carbon and bromine isotope fractionation; (ii) validate the model with the isotopic data observed during EDB degradation reactions; (iii) illustrate the capabilities of the model based on scenarios of complex EDB degradation pathways, including multistep reactions and parallel degradation pathways, and considering the evolution of dual C and Br isotope signals not only of EDB but also of its degradation intermediates and products.

2. Modelling approach

2.1. Degradation pathways and reaction mechanisms

We focus on EDB degradation through two important degradation pathways, dibromoelimination and nucleophilic substitution (S_N2). The two degradation pathways can occur both chemically and biotically. Dibromoelimination occurs during reduction of EDB with Zn(0) in aqueous solution, as well as during biotic transformation by *Sulfurospirillum multivorans*. A stepwise nucleophilic substitution may take place in aqueous alkaline solution and also occurs during biotic transformation by *Ancylobacter aquaticus* [24]. The two pathways involve different bond-cleavage mechanisms. Dibromoelimination is assumed to result in simultaneous cleavage of two C-Br bonds, while S_N2 reaction follows a stepwise cleavage of one C-Br bond [5,24].

93 ***2.2. Pathway-specific reaction rates and isotope fractionation***

94 In order to simulate carbon and bromine isotopic evolution of EDB via different reactions, we track
95 dual element isotopologues. The relative abundances of such isotopologues can be computed
96 considering the occurrence of both stable carbon and bromine isotopes:

$$A_j = \binom{2}{a} \cdot X^a \cdot (1-X)^{2-a} \cdot \binom{2}{b} \cdot Y^b \cdot (1-Y)^{2-b} \quad (1)$$

97 where A is the relative abundance of the j^{th} EDB isotopologue containing a ^{13}C out of a total of two
98 carbon atoms and b ^{81}Br out of a total of two bromine atoms. X and Y are the abundance of heavy
99 carbon and bromine isotopes, respectively.

100 Position specific fractionation factors for the j^{th} EDB isotopologue can be calculated according to
101 the corresponding apparent kinetic isotope effect (AKIE) derived from the observed bulk
102 enrichment factors:

$$\alpha_{rp,C} = AKIE_C^{-1} \approx 1 + \frac{2}{z_C} \cdot \varepsilon_C \quad (2)$$

$$\alpha_{rp,Br} = AKIE_{Br}^{-1} \approx 1 + \frac{2}{z_{Br}} \cdot \varepsilon_{Br} \quad (3)$$

103 where α_{rp} is the fractionating factor at reactive position, ε is bulk enrichment factor, and z is the
104 number of carbon or bromine atoms at reactive positions. In this work we calculated α_{rp} based on
105 the AKIE values reported in Kuntze et al. (2016); however, α_{rp} could be also derived by fitting the
106 proposed model to the raw isotope data.

107 We track the concentration change of each isotopologue considering a specific kinetic rate law. To
108 illustrate the approach, a first-order kinetic formulation is considered in the following equations;
109 however, as discussed for the application examples in Section 3, any degradation rate can be
110 implemented, including Michaelis-Menten kinetics. Since different reaction mechanisms of EDB

111 involve carbon and bromine atoms located at different isotopically-sensitive positions, the reaction
112 rates have to take into account all the fractionating atoms. Concerning the dibromoelimination
113 reaction, the two C-Br bonds are cleaved simultaneously, and thus the reaction rate for a specific
114 carbon-bromine isotopologue is given as:

$$r_j = k \cdot C_j \cdot (\alpha_{rp,C})^a \cdot (\alpha_{rp,Br})^b \tag{4}$$

115 where r_j is the reaction rate for the j^{th} isotopologue, k is the first-order reaction rate constant, C_j is
116 the concentration of the j^{th} isotopologue, α_{rp} is the fractionation factor as defined in Eqs. (2) and (3).

117 Considering S_N2 reaction of EDB, such reaction pathway involves the cleavage of one single C-Br
118 bond. In this case the reaction rate of EDB depends on the isotopic composition of the C-Br bond
119 that is cleaved, and thus the reaction rate of the individual isotopologues is defined in a bond-
120 specific manner as previously proposed for the carbon-chlorine isotope modelling of chlorinated
121 ethenes [30]. Due to the fact that the two C-Br bonds of EDB are chemically equivalent for S_N2
122 reaction, the approach is based on isotopologues without the need of specifying individual
123 isotopomers. Thus, the reaction rate, r_j, for a given j^{th} EDB isotopologue is expressed as the sum of
124 the following bond-specific reaction rates:

$$r_{j,^{12}C-^{79}Br} = k \cdot C_j \cdot \frac{(2 - n_{rp,^{13}C})}{2} \cdot \frac{(2 - n_{rp,^{81}Br})}{2} \tag{5}$$

$$r_{j,^{13}C-^{79}Br} = k \cdot C_j \cdot \alpha_{rp,C} \cdot \frac{n_{rp,^{13}C}}{2} \cdot \frac{(2 - n_{rp,^{81}Br})}{2} \tag{6}$$

$$r_{j,^{12}C-^{81}Br} = k \cdot C_j \cdot \alpha_{rp,Br} \cdot \frac{(2 - n_{rp,^{13}C})}{2} \cdot \frac{n_{rp,^{81}Br}}{2} \tag{7}$$

$$r_{j,^{13}C-^{81}Br} = k \cdot C_j \cdot \alpha_{rp,C} \cdot \alpha_{rp,Br} \cdot \frac{n_{rp,^{13}C}}{2} \cdot \frac{n_{rp,^{81}Br}}{2} \tag{8}$$

$$r_j = \sum_{i=1}^{N} r_{j,(C-Br)_i} \tag{9}$$

where k is the first-order rate constant, C_j is the concentration of the j^{th} isotopologue, and n_{rp} represents the total number of reactive carbon or bromine atoms within the isotopologue, i indicates the C-Br bond cleaved during the reaction and N is the total number of C-Br bonds that can be cleaved for the j^{th} EDB isotopologue. Note that an overall rate for all isotopologues can be computed from the rate of each isotopologue as $\sum_{i=1}^{m} r_j$, where m is the total number of isotopologues.The concentration change of the j^{th} isotopologue of EDB is described as:

$$\frac{dC_j}{dt} = -r_j \tag{10}$$

The total concentration of EDB can be obtained by summing the concentrations of each isotopologue:

$$C_{tot} = \sum_{j=1}^{m} C_j \tag{11}$$

where C_{tot} is the total concentration of EDB, C_j is the concentration of the j^{th} isotopologue and m is the total number of EDB isotopologues.

The concentration of each isotopologue is used to calculate stable carbon and bromine isotope ratios by considering the total number of heavy and light isotopes [14] and are expressed as:

$$R_C = \frac{Tot(^{13}C)}{Tot(^{12}C)} = \frac{\sum_{j=1}^{m} a \cdot C_j}{\sum_{j=1}^{m} (2-a) \cdot C_j} \tag{12}$$

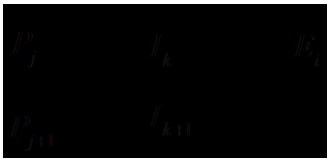
$$R_{Br} = \frac{Tot(^{81}Br)}{Tot(^{79}Br)} = \frac{\sum_{j=1}^{m} b \cdot C_j}{\sum_{j=1}^{m} (2-b) \cdot C_j} \quad (13)$$

where R_C and R_{Br} are the carbon and bromine isotope ratios of EDB, C_j is the concentration of the j^{th} isotopologue, m is the total number of EDB isotopologues as defined in Eq (11), a and b are the number of heavy carbon and heavy bromine isotopes, as defined in Eq (1).

2.3. Complex Reaction Pathways

In the previous section we illustrated the modelling for single step reactions. However, the model can be applied also when degradation occurs through more complex reaction pathways involving sequential and parallel reactions. In this cases, if one aims at describing the formation and consumption of intermediates and products and the evolution of their dual-element isotopic composition, it is necessary to take into account that a given intermediate (or product) can be formed by two distinct isotopologues of the parent compound (or intermediate).

Sequential multistep reactions. We consider EDB degradation through sequential multistep reactions, specifically, through a reaction pathway involving two S_N2 type reactions. As parent compound (P) the two different EDB isotopologues considered are: the j^{th} isotopologue and the $(j+1)^{th}$ isotopologue. The latter contains one more ^{81}Br isotope in the molecule compared to the j^{th} isotopologue. The parent compound (P) is sequentially degraded into the k^{th} and $(k+1)^{th}$ isotopologues of the intermediate (I), and finally into the i^{th} isotopologue of the end product (E), which is completely debrominated. The two-step reaction can be illustrated as:



(14)

Note that the different letters used as subscripts indicate that the different compounds may have a different number of isotopologues.

158 The concentration of the isotopologues of the parent compound (*P*), the intermediate (*I*) and the end
159 product (*E*) are described:

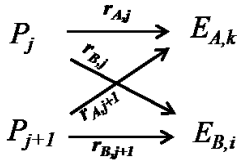
$$\frac{dP_j}{dt} = -r_{1,j} \tag{15}$$

$$\frac{dI_k}{dt} = +r_{1,j} + r_{1,j+1} - r_{2,k} \tag{16}$$

$$\frac{dE_i}{dt} = +r_{2,k} + r_{2,k+1} \tag{17}$$

160 where $r_{1,j}$, $r_{1,j+1}$, $r_{2,k}$ and $r_{2,k+1}$ are the isotopolgue-specific reaction rates for the parent compound
161 and for the intermediate, respectively. The kinetic formulation for such reaction rates $r_{1,j}$ and , $r_{1,j+1}$
162 (parent compound), as well as $r_{2,k}$ and $r_{2,k+1}$ (intermediate) are based on Eqs. 5-9. The carbon and
163 bromine isotope ratios for the parent compound, intermediate and end product can be calculated
164 according to Eqs. 12-13.

165 Parallel reactions. Competition between different reaction pathways of EDB degradation has been
166 observed in several experimental studies [5,24]. We consider the case of EDB transformation
167 through two competing reaction pathways that yield two different products:



168 (18)

169 The concentration of the j^{th} isotopologues of the parent compound (P_j) and the k^{th} and i^{th} of the two
170 end products ($E_{A,k}$ and $E_{B,i}$) are given as:

$$\frac{dP_j}{dt} = -r_{A,j} - r_{B,j} \tag{19}$$

$$\frac{dE_{A,k}}{dt} = +r_{A,j} + r_{A,j+1} \quad (20)$$

$$\frac{dE_{B,i}}{dt} = +r_{B,j} + r_{B,j+1} \quad (21)$$

where $r_{A,j}$, $r_{A,j+1}$, $r_{B,j}$ and $r_{B,j+1}$ are the reaction rates for the individual reaction pathway of the j^{th} and $(j+1)^{th}$ isotopologue of the parent compound (P).

2.4. Model implementation

The governing equations describing the simultaneous evolution of the concentrations, as well as the carbon and bromine isotope ratios are implemented in MATLAB®. The system of ordinary differential equations is solved numerically using the function *ode15s*. The experimental data and the key isotope fractionation parameters are taken from the experimental work of Kuntze et al. (2016); the latter are summarized in Table 1. The simulation was run for a time covering the duration of the experiments (i.e., 4 hours for the two cases of dibromoelimination by both Zn(0) and *S. multivorans*, 350 hours for abiotic degradation in alkaline solution and 8 hours in the case of biotic degradation by *A. aquaticus*). The EDB concentration data were used to determine the kinetic parameters of the degradation rates. First-order and Michaelis-Menten kinetics were considered for the abiotic and biotic reaction pathways, respectively. A fitting procedure, minimizing the sum of normalized squared errors based on the function *lsqnonlin,* was used to obtain the values of the kinetic parameters. As illustrated above, the proposed approach tracks the dual-element EDB isotopologues. Nine EDB isotopologues were considered in the simulations by taking into account all possible combinations of carbon and bromine isotopes. The abundances of these isotopologues were determined based on Eq. 1.

[insert Table 1 here]

3. Results and discussion

3.1. Chemical and biotic dibromoelimination reactions

The dual carbon and bromine isotope approach has been used to investigate EDB degradation by dibromoelimination reactions [24]. To reproduce the experimental data observed during dibromoelimination reactions, we simulated carbon and bromine isotopic evolution according to the hypothesized two-electron transfer dibromoelimination mechanism. The simulation results (solid lines in Fig. 1) are shown together with the reported experimental data (symbols in Fig. 1). A first-order kinetic (k=0.9 h^{-1}) is used to describe the concentration variation of EDB during dibromoelimination by Zn(0) (Fig. 1a), where the concentration decreases down to 4.3% of the initial concentration value. The model also accurately predicts the carbon and bromine isotope fractionation (Fig. 1b), which are simulated based on the experimentally evaluated *AKIE* values ($AKIE_C$=1.0223, $AKIE_{Br}$=1.0042) [24]. The results show different extents of carbon and bromine fractionation, with $\delta^{13}C$ values changing from -26.3‰ to -3.8‰ and $\delta^{81}Br$ varying from 0.5‰ to 5.4‰. For biotic dibromoelimination, a Michaelis-Menten kinetics, with maximum degradation rate k_{max}=0.2289 mmol·L^{-1}·h^{-1} and half-saturation constant K_s=0.0166 mmol·L^{-1}, is used in our model to reproduce the observed concentration data during biotic dibromoelimination by *S. multivorans* (Fig. 1c). The carbon and bromine AKIE values of 1.0107 and 1.0046 are used in the model to describe carbon and bromine isotope effects. The fractionation was introduced in the maximum degradation rate and led to an increase of 12.7‰ for ^{13}C and of 6.2‰ for ^{81}Br isotopes during degradation of about 95% of the initial EDB concentration.

[insert Figure 1 here]

Linear dual carbon-bromine isotopic trends with different slopes are obtained for chemical (slope of 5.3) and biotic (slope of 2.4) dibromoelimination reactions and the model accurately captures the two different dual-isotope trends. The excellent agreement between experimental and modelling

results demonstrates the capability of the proposed mechanistic model to simultaneously capture the evolution of both concentration and carbon-bromine stable isotopes.

3.2. Chemical and biotic nucleophilic substitution (S_N2) reactions

Nucleophilic substitution (S_N2) reaction is another important degradation mechanism for EDB. A stepwise scenario is followed by both biotic and chemical S_N2 reactions, where the cleavage of one carbon-bromine bond of EDB is hypothesized as the isotopically sensitive step. We provide a model-based interpretation of the experimental data provided in the study of Kuntze et al. [24], who observed carbon and bromine isotope fractionation of EDB during chemical degradation in aqueous alkaline solution as well as during biotic S_N2 reaction by *Ancylobacter aquaticus*.

[insert Figure 2 here]

We use a first-order kinetics (k=0.0125 h^{-1}) to describe the concentration change during the chemical S_N2 reaction (solid line in Fig. 2a). The corresponding carbon isotope ratio varies from -11.6‰ to 93.8‰, and the bromine isotope fractionation occurs in a range between 0.4‰ and 4.3‰. The biotic S_N2 reaction of EDB is described by a Michaelis-Menten kinetics with maximum degradation rate (k_{max}=0.4329 mmol·L^{-1}·h^{-1}) and half-saturation constant (K_s=0.0288 mmol·L^{-1}) evaluated based on the observed concentration data (Fig. 2c). The simulation of carbon and bromine isotope signals is based on the reported *AKIE* values ($AKIE_C$=1.062 and $AKIE_{Br}$=1.002 for the chemical S_N2 reaction; $AKIE_C$=1.014 and $AKIE_{Br}$=1.0012 for the biotic S_N2 reaction). The simulations of the biotic and chemical S_N2 transformations of EDB were able to capture the different fractionation of the two reaction pathways observed in the experiments. Specifically, the biotic transformation resulted in a smaller extent of both carbon and bromine isotope fractionation (17.9‰ for $\delta^{13}C$ and 1.8‰ for $\delta^{81}Br$) whereas the chemical S_N2 reaction resulted in stronger fractionation (105.4‰ for $\delta^{13}C$ and 3.9‰ for $\delta^{81}Br$).

238

239 Fig. 3 summarizes the dual element isotope plots for the four cases of chemical and biotic EDB
240 degradation through dibromoelimination and S_N2 type nucleophilic substitution. The four different
241 reactions are adequately characterized in the dual carbon-bromine isotope plot. In all the cases the
242 simulation outcomes closely reproduce the experimental results. Note that these outcomes are no
243 linear fits of the experimental data, but represent mechanistic descriptions of EDB degradation
244 through different reaction pathways according to the approach outlined in Section 2. For all
245 considered cases of EDB degradation the normalized root mean squared error was calculated as a
246 quantitative measure of the goodness-of-fit. Such metric was computed for both carbon and
247 bromine isotope data and yielded values in a range of 0.036-0.158 for carbon and 0.068-0.16 for
248 bromine. The successful comparison of the simulation results with the experimental data highlights
249 the capability of the proposed approach to quantitatively describe different mechanisms of EDB
250 degradation.

251 **[insert Figure 3 here]**

252 ***3.3. Scenario modelling***

253 Based on the validated model presented above, we also investigated scenarios involving complex
254 EDB reaction pathways, such as sequential multistep reactions (Scenario 1 in Fig. 4) and parallel
255 reactions (Scenario 2 in Fig. 4). In the examples illustrated in the previous section and in most
256 experimental studies, the CSIA approach has been mainly focusing on the parent compound.
257 However, stable isotope analysis of reaction products can also be very informative about the
258 underlying reaction steps characterizing different reaction mechanisms [22,31]. To explore the
259 potential of carbon and bromine CSIA of EDB degradation products, we simulate the evolution of
260 the concentration and the isotopic signals of the parent compound, the intermediates and the end

products for the two proposed reaction scenarios illustrated in Fig. 4: 1) multistep S_N2 nucleophilic substitution and 2) simultaneous occurrence of the S_N2 reaction and dehydrobromination.

[insert Figure 4 here]

The S_N2 reaction involves the stepwise cleavage of two C-Br bonds, kinetic isotope effects for C-Br cleavage (KIE_C=1.042; KIE_{Br}=1.002) were calculated in the previous experimental study based on the Streitwieser limit [24]. Since isotope fractionation of the intermediate has not been experimentally determined (yet), the theoretical *KIEs* values are used as model input parameters to differentiate the reaction rates of the different carbon-bromine isotopologues of both parent and intermediate compounds. The simulation results for sequential multistep EDB degradation (Scenario 1) are shown in Fig. 5. The degradation of the parent compound EDB (blue solid line in Fig. 5a) results in the formation of the intermediate, bromoethylene glycol (red dotted line, k=0.5 h^{-1}), which is further transformed to ethylene glycol (green dash-dotted line, k=2.5 h^{-1}) that accumulates as the end product. The temporal carbon and bromine isotope trends are reported in Fig. 5b and 5c and show a linear increase of $\delta^{13}C$ and $\delta^{81}Br$ values for EDB. However, the increasing trends of carbon and bromine isotope ratios become nonlinear for the intermediate, bromoethylene glycol. This is due to the fact that bromoethylene glycol (red dotted line) further degrades and preferentially transfers ^{12}C isotopes to the end product and meanwhile preferentially releases ^{79}Br during its transformation. As a result, the $\delta^{13}C$ values of the end product, ethylene glycol (green dash-dotted line), continuously increase and approach the original carbon isotope signature of EDB. In the dual carbon-bromine isotope plot (Fig. 5d) EDB and bromoethylene glycol have different trends. EDB shows a linear increase with a slope of 20.2, whereas a nonlinear curve, with a slope varying from 11 to 21.5, describes the trend of bromoethylene glycol. This nonlinear behaviour is due to the simultaneous formation and consumption of bromoethylene glycol, which occur at different rates and involve different extents of carbon and bromine isotope fractionation

285 during the course of the degradation reaction. For multistep reactions with formation and further
286 degradation of intermediates, a mechanistic modelling approach is helpful since it allows the
287 simultaneous interpretation of isotope fractionation for both precursors and reaction products.

288 **[insert Figure 5 here]**

289 In Scenario 2, degradation of EDB in alkaline solution is considered. In this scenario two competing
290 reaction pathways, i.e., nucleophilic substitution (S_N2) and dehydrobromination, occur
291 simultaneously. Concerning dehydrobromination, this reaction pathway involves the simultaneous
292 cleavage of a carbon-bromine bond and a carbon-hydrogen bond. The theoretical carbon and
293 bromine isotopic effects during cleavage of C-H (KIE_C=1.021) and C-Br (KIE_C=1.042; KIE_{Br}=1.002)
294 bonds [24,36] are considered to calculate the fractionation factors at reactive positions used in the
295 isotopologue-specific rate expression (Eq. 4). We applied the model to simulate concentrations and
296 isotope ratios for such a parallel reaction system. We assume that the two reactions follow a first-
297 order kinetic with rate constants of 0.5 h^{-1} and 0.03 h^{-1} for S_N2 reaction and dehydrobromination,
298 respectively. These values were selected according to the relative contribution of 93% (sequential
299 S_N2 reaction) and 7% (dehydrobromination) observed in the experimental study of Kuntze et al.
300 [24]. The evolution of concentration, carbon and bromine isotopic signals is simultaneously
301 simulated for EDB, the intermediate and the end products. As shown in Fig. 6a, the two competing
302 degradation reactions cause a decrease of the EDB concentration (blue solid line). The intermediate,
303 bromoethylene glycol (red dotted line), is formed and further degrades into ethylene glycol (green
304 dash-dotted line) by nucleophilic substitution (S_N2). In parallel, dehydrobromination causes the
305 formation of vinyl bromide (black solid line). Fig. 6b illustrates the $\delta^{13}C$ trends for the species
306 involved in the two reaction pathways: the parent compound shows a linear behaviour, whereas the
307 intermediate and the end products show nonlinear curves with decreasing slope. Bromine stable
308 isotope ratios are shown in Fig. 6c. Stable bromine isotope fractionation occurs at different extents

for the two brominated degradation products: $\delta^{81}Br$ is enriched by 7.6‰ for bromoethylene glycol (S_N2 reaction) and by 1‰ for vinyl bromide (dehydrobromination), because the former is an intermediate which undergoes further debromination, whereas vinyl bromide represents a final product in this scenario. In the dual-isotope plot (Fig. 6d) a linear trend is obtained for the parent compound EDB (slope: 21.2), as well as for vinyl bromide from the dehydrobromination reaction (slope: 20.3). A nonlinear dual-isotope trend is obtained for the S_N2 reaction intermediate, bromoethylene glycol, with a slope varying from 11.1 to 22.9. The dual isotope trend of EDB (blue line in Fig. 6d) appears very similar with the one obtained during 100% S_N2 reaction (lower dashed line). This is due to the fact that, in the considered scenario, the S_N2 reaction is the dominant pathway (about 93% contribution) during EDB degradation in alkaline solution. The shaded grey area between the dotted lines indicates the possible range for the investigated scenario: from 100% contribution of dehydrobromination (upper bound) to 100% contribution of the S_N2 reaction (lower bound). The dual element isotope signatures of the reaction products from the different pathways have a distinct behaviour and are different from the trend of the parent compound. The simulation results indicate that in practice it might be difficult to accurately quantify the contribution of each concurrent reaction pathway exclusively based on the observed EDB carbon and bromine isotope data. However, these simulations also demonstrate that CSIA of reaction intermediates and end products can bring new possibilities to elucidate the underlying reaction steps and to accurately quantify the contributions of individual reaction pathways.

[insert Figure 6 here]

4. Conclusion

Dual carbon-bromine isotope investigation significantly improves the understanding of various reaction mechanisms of brominated organic compounds. Recent studies have focused on the

development of analytical techniques for bromine CSIA as well as experimental investigation of degradation mechanisms of different brominated organic pollutants. In this study, we have proposed an integrated modelling approach allowing the simultaneous prediction of concentrations and dual-element isotope ratios. Our investigation focused on carbon and bromine isotope fractionation during 1,2-dibromoethane (EDB, ethylene dibromide) transformation through different reaction pathways. The proposed modelling approach tracks dual-element isotopologues. The method allowed us reproducing the carbon and bromine isotopic signal observed in experimental studies of different chemical and biotic EDB reaction pathways. The approach is based on bond-specific reaction rates and can be readily extended to cases in which different contaminants and/or reaction pathways will require tracking also isotopomers (i.e., molecules with the same number of each isotopic atom but differing in their position). Furthermore, we exemplified the model capabilities with two scenarios involving complex reaction systems with sequential and parallel reactions, respectively. In the considered case of multistep nucleophilic substitution (S_N2) reaction, concentration and isotopic ratios of the parent compound, the intermediate and the end product were predicted based on the validated model. Different carbon and bromine isotopic behaviours of the parent compound and intermediate were obtained. In the scenario modelling of EDB degradation via two concurrent reactions, our simulation results showed that stable isotope analysis of the reaction products is beneficial, since it allows quantifying further degradation of the intermediate product in multistep reactions, as well as providing a more accurate evaluation of the individual contributions of different concurrent pathways to the overall reaction. This is particularly beneficial when one of the reaction pathways is dominant and, therefore the sole analysis of the dual isotope trend of the parent compound would not be conclusive in identifying which degradation reactions are responsible for the contaminant degradation. The proposed model was applied to the specific case of EDB degradation, however it provides a framework that can be extended to other

brominated compounds that may undergo degradation through different reaction pathways. The first-principle based formulation of the approach will also facilitate future model-based applications in complex environmental systems, in which both transformation and mass transfer processes may affect the observed isotope signals.

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382 **Table 1.** Reaction mechanisms, bulk enrichment factors (ε_{bulk}) and fractionation factors at reactive position
383 (α_{rp}) for the different EDB degradation reactions.

Reaction	Mechanism	ε_{bulk}		α_{rp}	
		C	Br	C	Br
Zn (0)	dibromoelimination	-10.9±1.1	-2.1±0.3	0.9891±0.0011	0.9979±0.0003
S. multivorans	dibromoelimination	-5.3±0.5	-2.3±0.2	0.9947±0.0005	0.9977±0.0002
Alkaline solution	abiotic S_N2	-29.2±2.6	-1.0±0.1	0.9416±0.0052	0.9980±0.0002
A. aquaticus	biotic S_N2	-6.9±0.4	-0.6±0.1	0.9862±0.0008	0.9988±0.0002

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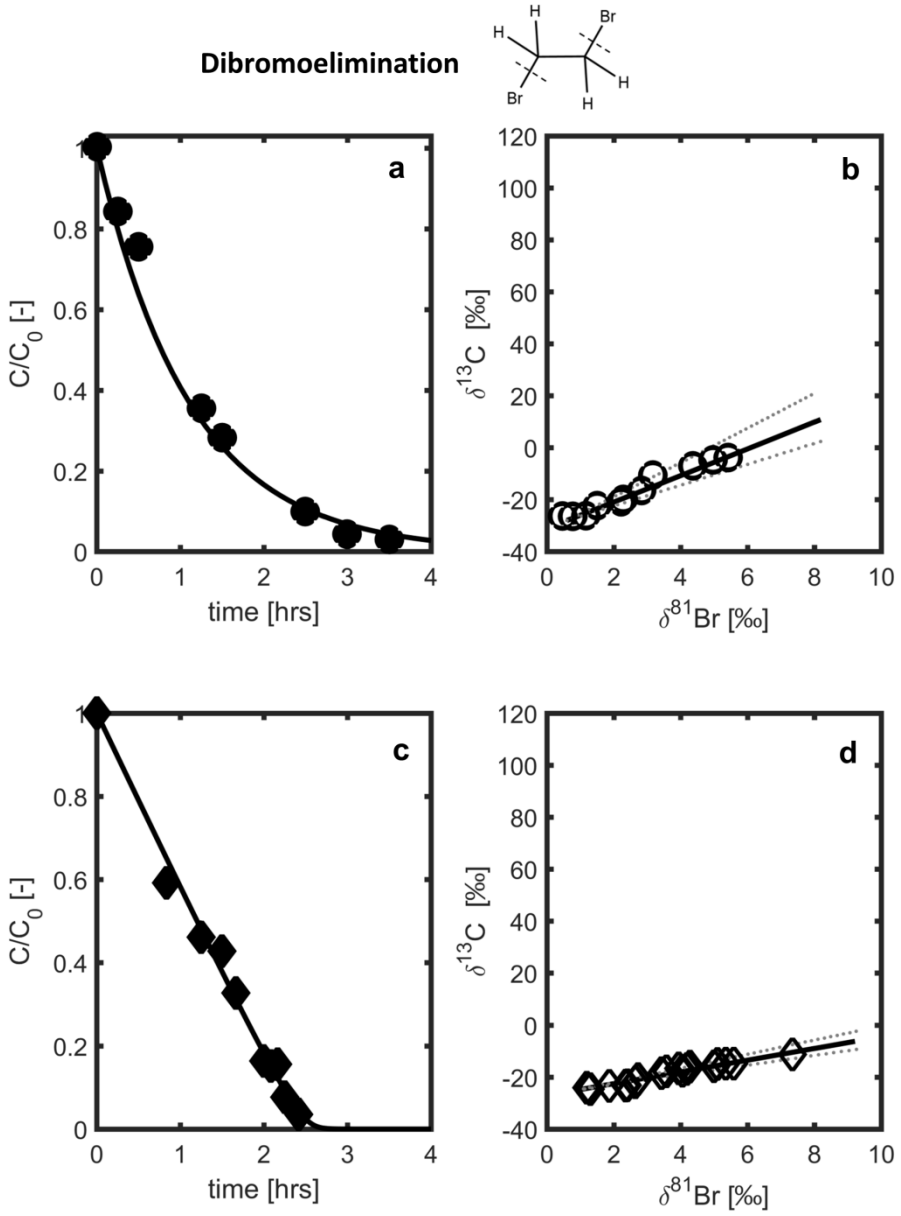
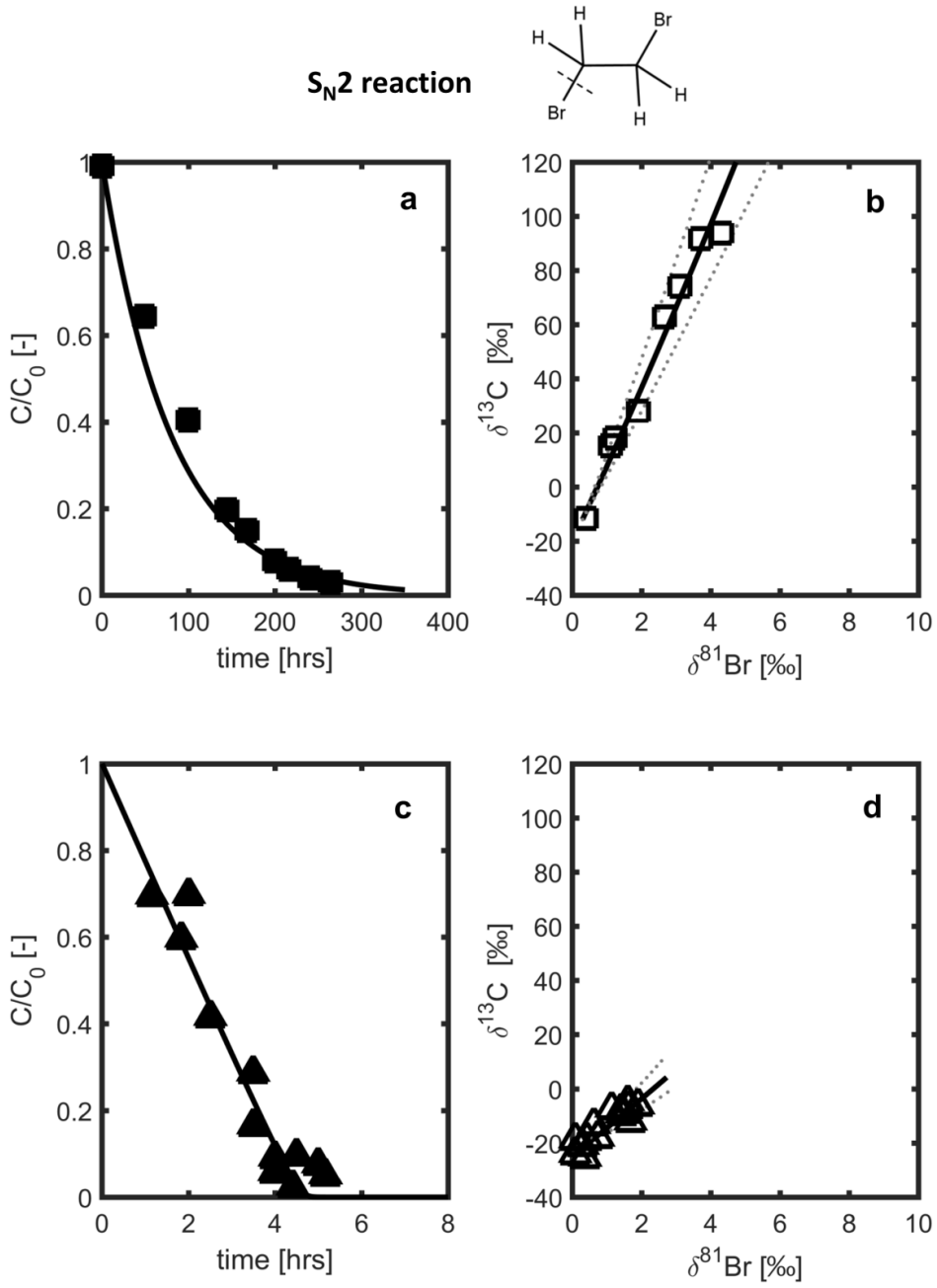


Figure 1. Concentration change and dual carbon-bromine isotope fractionation during dibromoelimination reaction by Zn(0) (Panels (a) and (b)) and biotic reaction with *S. multivorans* (Panels (c) and (d)). The symbols represent the experimental data reported in Kuntze et al. [24], and the solid lines are the simulation results.



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396 **Figure 2.** Carbon and bromine isotope fractionation during EDB chemical transformation in alkaline
397 solution and biotic reaction by *Ancylobacter aquaticus*. Panel (a) and (c): the symbols represent the observed
398 concentration profiles reported in Kuntze et al. [24], and the lines are the simulation results. Panel (b) and (d):
399 the symbols are carbon-bromine isotopic data and the solid lines are the simulation results.

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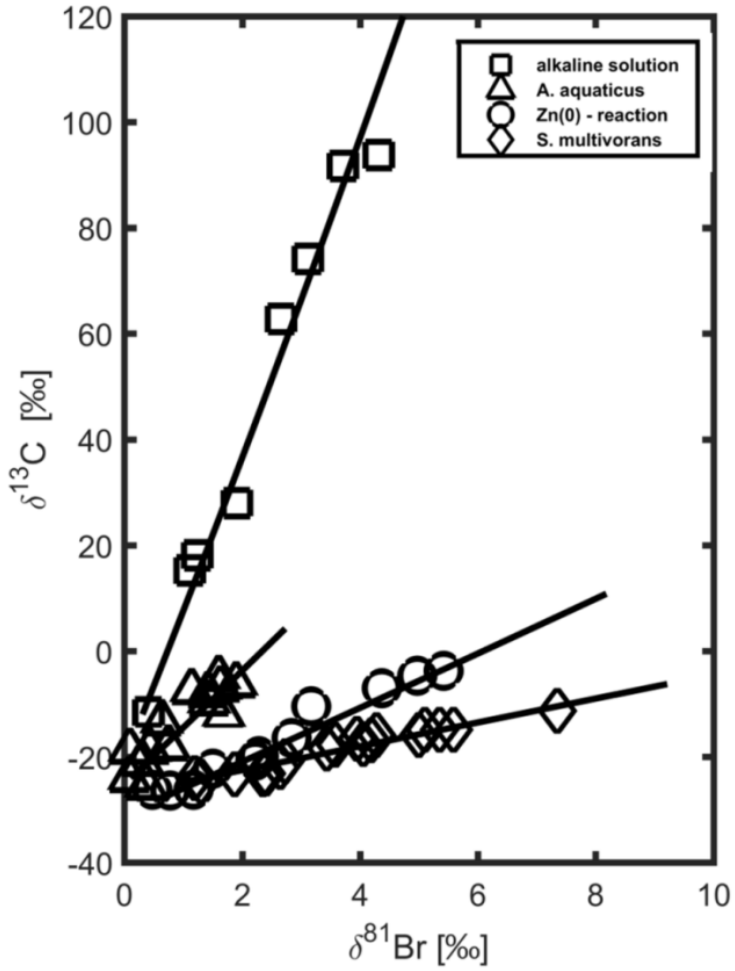
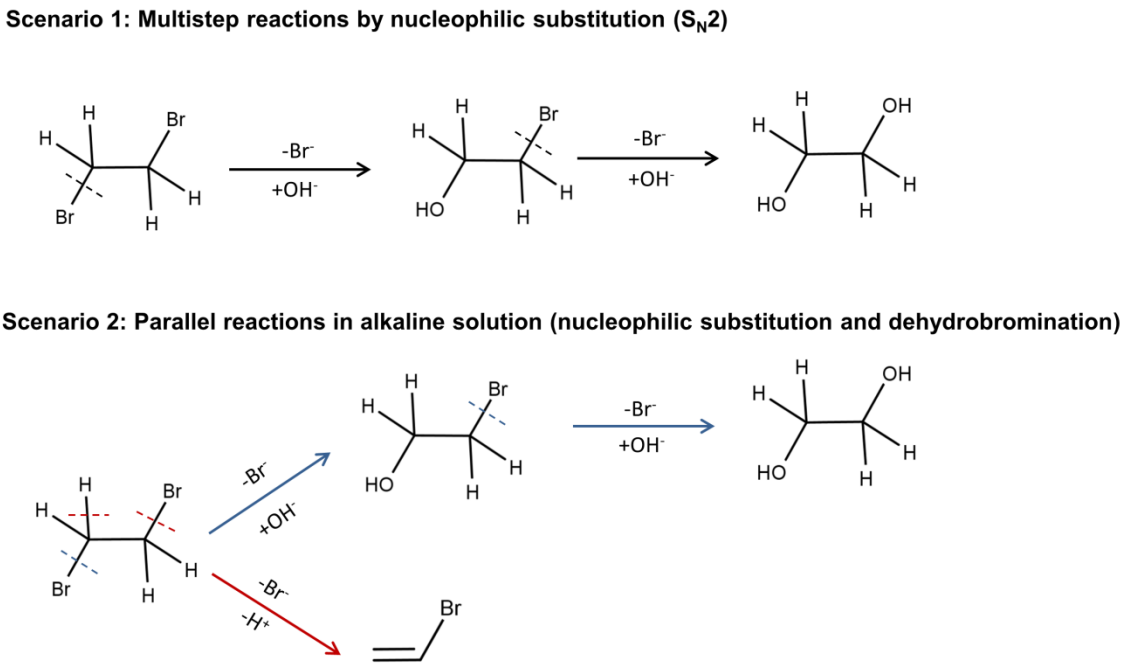


Figure 3. Carbon and bromine isotope fractionation for different EDB degradation reactions. The symbols represent the experimental data reported in Kuntze et al.[24], and the solid lines represent the results of the simulations corresponding to 99% degradation of the initial EDB concentration.



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413 **Figure 4.** Schemes representing the sequential and parallel reaction pathways considered in the scenario

414 modelling.

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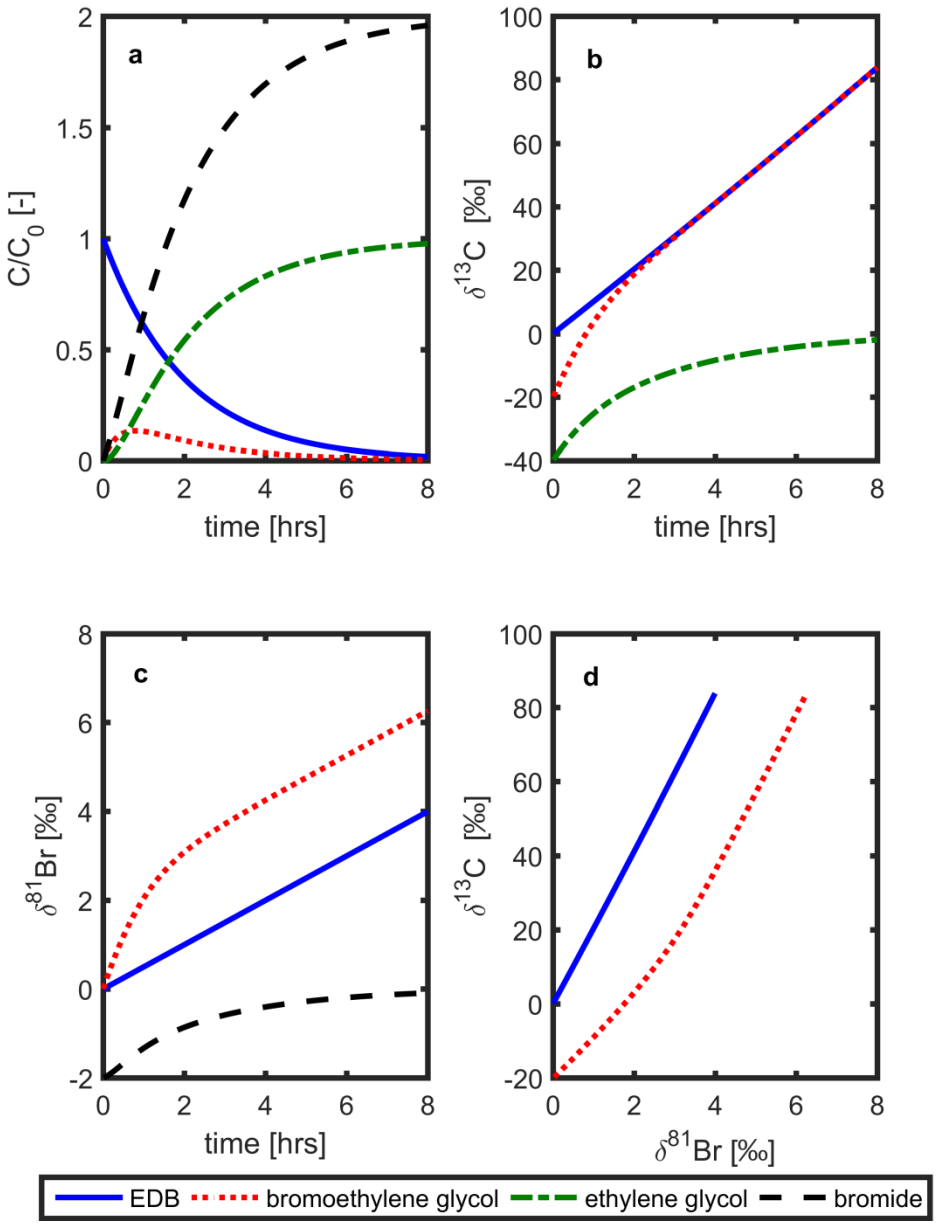

Figure 5. Concentration, carbon and bromine isotope fractionation of EDB, the intermediate (bromoethylene glycol) , the end product (ethylene glycol) and bromide during multi-step nucleophilic substitution (Scenario 1 in Fig. 4).

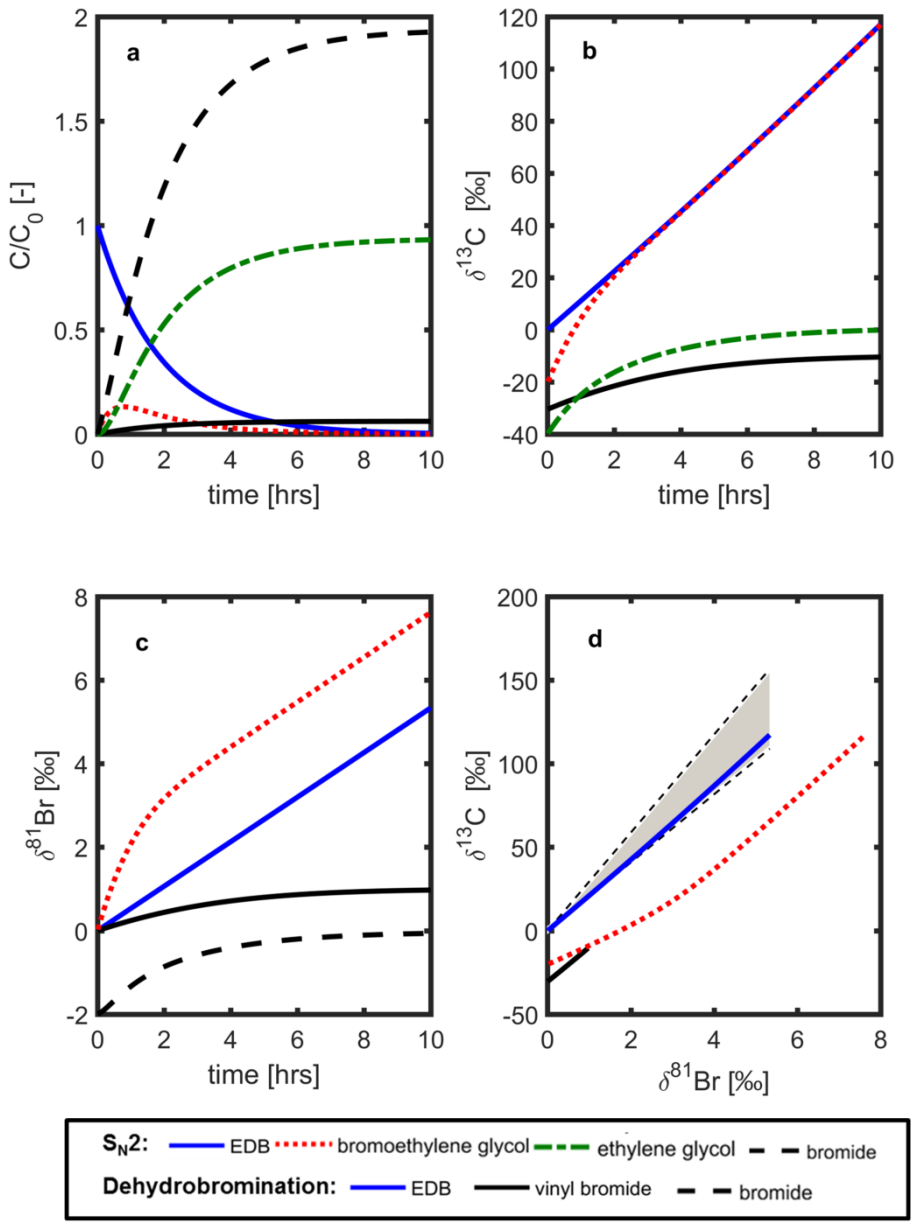

Figure 6. Concentration, carbon and bromine isotope fractionation of EDB during the two competing reaction pathways: nucleophilic substitution (S_N2) reaction and dehydrobromination (Scenario 2 in Fig. 4). The shaded area in Panel (d) indicates EDB dual-isotope trends corresponding to different contributions of each reaction pathway considered in Scenario 2. The dotted lines on the upper and lower bounds represent dual-isotope trends of EDB degradation when one reaction pathway occurs exclusively (i.e., 100% dehydrobromination and 100% S_N2 reaction, respectively).

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